Vaccination of fish
Present status and future challenges

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Background - aquaculture

- Agriculture and fisheries will soon reach the maximum capacity in global food production
- Consequently, food from aquaculture will be more important for future food security
- Aquaculture must be sustainable with minimal use of antibiotics and chemicals
- Correct use of vaccines will contribute to sustainability in aquaculture

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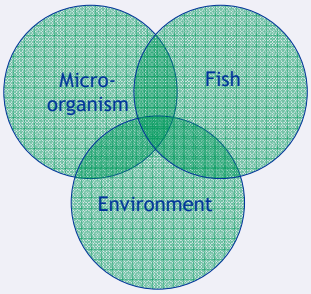
The health triangle: relationship of the micro-organism, the fish and the environment



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Background - vaccines

- The first vaccines for aquaculture were simple products like formalin inactivated bacterial cultures
- Most modern fish vaccines are research based products
- The way from research to licenced vaccines is long and expensive
- A licenced vaccine may be required for import of aquaculture products
- Many countries (EU-countries and USA) and organisations (FAO, OIE, WTO) have strict import requirements related to the use of vaccines in aquaculture production

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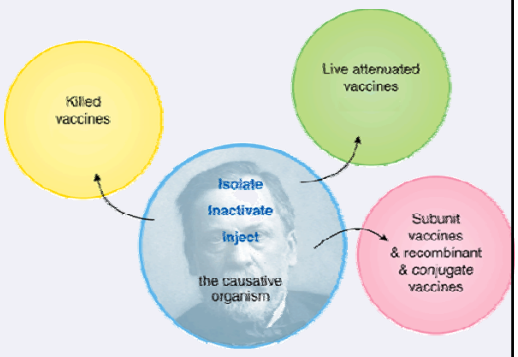
From Louis Pasteur to fish vaccinology



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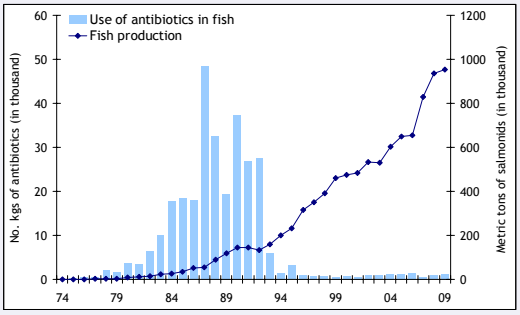
Use of antibiotics and production of salmonid fish in Norway



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Immune system of fish

Similarities and differences compared with warm blooded animals

- Innate immunity is more important in fish
- Specific immunity is less developed in fish
  - Maternal immunity cannot be used for disease prevention in fish
- Protective immunity can be developed even in young fish
  - Salmonid fish at a size of less than 5 grams develop immunity
- Variation between fish species
  - Salmonid fish differ from cod fish

Diseases in fish

The pathogenicity of fish microorganisms is species specific

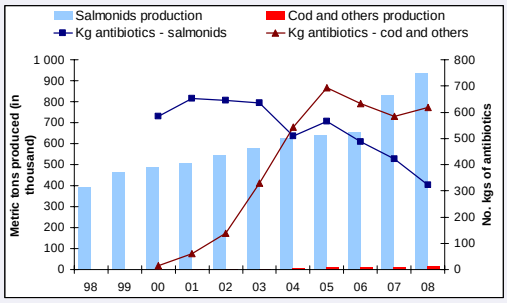
Examples:

- Different serotypes of *Vibrio anguillarum* have different virulence for Atlantic salmon, rainbow trout and cod
- Furunculosis in Atlantic salmon is caused by *Aeromonas salmonicida* subsp *salmonicida*, whereas furunculosis in cod is caused by *A. salmonicida* subsp *achromogenes*
- Viral haemorrhagic septicaemia-virus (VHS) may give high mortality in rainbow trout and low or no mortality in Atlantic salmon

Conclusion:

- Vaccines must be tailor made for a certain fish species based on disease surveillance of the population

Use of antibiotics and production of fish



Successful vaccination of fish

	Successful vaccination
Bacterial diseases	Several
Viral diseases	Uncertain
Fungal diseases	None
Parasite diseases	None

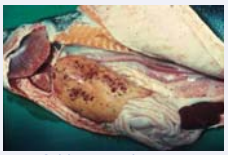
Successful vaccination against bacterial diseases

Atlantic salmon and rainbow trout are vaccinated against:

- vibriosis (*Vibrio anguillarum*)
- cold water vibriosis (*Vibrio salmonicida*),
- furunculosis (*Aeromonas salmonicida*)

prior to sea transfer with an injectable adjuvanted vaccine

Håstein et al. 2005 Dev.Biol 121, 55-74



Cold water vibriosis

Furunculosis

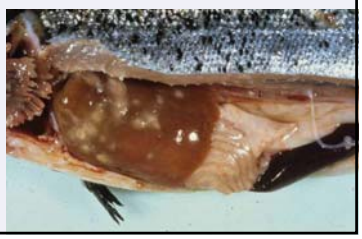
Successful vaccination against bacterial diseases cont.

- Sea bass and sea bream are vaccinated against pasteurellosis and turbot against streptococcosis with inactivated vaccines
- Live vaccines are used for vaccination against infections with *Edwardsiella ictaluri* in catfish and *Renibacterium salmoninarum in* Atlantic salmon

Håstein et al. Dev.Biol 2005, 121, 55-74
Shoemaker et al. J world aquac soc, 2009,40,573-585

Bacterial diseases cont.

No vaccines with acceptable protection against piscirickettsiosis in Atlantic salmon



Viral vaccines

- Inactivated vaccines are used against infectious pancreatic necrosis (IPN), infectious salmon anaemia (ISA) and pancreas disease (PD) with some effect
- A DNA vaccine against infectious haematopoietic necrosis (IHN) is licenced in Canada. The vaccine gives acceptable protection
- Research on DNA-vaccines for several diseases (IPN, VHS, koi herpes virus-infection, spring viraemia in carp)

Biering et al. 2005 Dev.Biol 121, 97-113
Salonius et al. 2007 Current opinion in investigational drugs 8, 635-641

Future challenges

Inactivated vaccines
- Improvements of efficacy and safety
- Adjuvants

Intracellular bacteria and virus
- Live vaccines
- Oral vaccines

Parasites and fungi
- Pathogenesis, antigens and immune response

Molecular biology in production and research
- Licenced vaccines
- DNA-vaccines
- DIVA-vaccines
- Vaccine production without agent

Inactivated vaccines - improvement

- The efficacy and even safety of vaccines are dependent on the conditions during cultivation
- Composition of the growth media and temperature are among the crucial factors
- An example: *Vibrio salmonicida* destined for vaccine use in fish should be cultured in an iron limited growth medium and at a temperature below 10° C.

Colquhoun DJ & Sørum H. Microb. Pathogenesis 2001;31:213-219

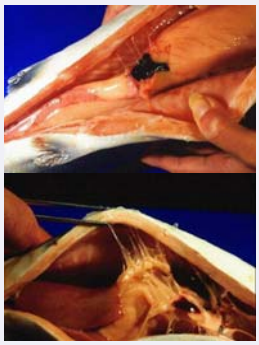
Adjuvants - a challenge

Adjuvants are necessary in order to achieve acceptable protection against some diseases

Oil adjuvants may cause adherence in peritoneal cavity and muscle pigmentation

The side effects caused by some adjuvants are not acceptable from an animal welfare point of view

The search for improved adjuvants and factors contributing to the side effects is a great challenge



Inactivated versus live vaccines

Inactivated vaccines

- Stimulate mainly humoral immunity
- Adjuvants may be required
- Administration by injection or immersion
- Generally safe, but may cause local reactions
- Relatively expensive

Inactivated versus live vaccines
Live vaccines

- Stimulate both humoral and cellular immunity
- Adjuvant is not required
- Immersion and oral administration
- Safety may be a concern
- Generally cheap to produce

Intracellular bacteria and virus

- Inactivated vaccines give no or insufficient protection
- Live attenuated vaccines for immersion or oral administration are the only way to achieve acceptable protection?

- Safe attenuation
  - Chemical methods
    - passage on media with rifampicin
  - Genetic engineering
    - deletion or insertion of genes

Shoemaker et al J World Aqua Soc 2009, 40, 573-580

Efficacious and safe vaccines

In order to make an efficacious and safe fish vaccine the following basic knowledge is crucial

Pathogenesis of the disease
- intracellular or extracellular

Critical antigens for protection
- adhesin, toxins, others

Immune response
- humoral and/or cellular

Vaccine against salmon louse

- The challenge is to identify concealed antigens from the gut of the louse and include them in a recombinant vaccine.
- The optimal antigens stimulate production of host antibodies binding to antigenic sites in the gut of the haematophagous louse
- Similar approach has been used with some success in the control of the cattle tick (*Boophilus microplus*)

Epizootic ulcerative syndrome (EUS) Is it possible to develop a vaccine?

- *Aphanomyces invadans* is difficult to grow in the laboratory
- Surface structures are believed to be virulence factors involved in the establisment and development of lesions
- A vaccine with antigens of virulence factors can be produced with modern techniques
- Cellular immunity is probably involved which may require live, attenuated vaccines
- The European community is supporting a project with the aim to develop a vaccine against *Saprolegnia* which is a similar oomycete causing losses in European aquaculture

Oral vaccines

- Few studies on oral vaccines in fish
- Most studies have been unsuccessful
- Oral vaccines require formulations that protect the antigen from inactivation and digestion in the stomach and anterior gut
- Different new approaches with microencapsulation or other ways of incorporation of antigens are promising
- Experimental oral vaccines against VHS (attenuated strain) and IPN (DNA-vaccines)

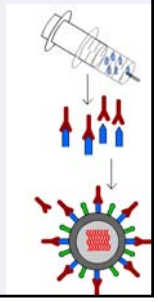
Adelmann et al Vaccine 2008
de las Heras et al. Fish Shellfish Immunol 2010,1-9

New technologies

- Sub-unit vaccines
- Deletion mutants
- Live vectored vaccines
- DNA-vaccines
- Pyro-sequencing

Subunit vaccines

- Subunit vaccines contain a portion of the infectious agent which is essential for stimulation of protective immunity
- Subunit fish vaccines are commercially available
- Example: IPN-vaccine for salmonid fish
- Advantage: Safe and inexpensive
- Disadvantage: No intracellular replication and inadequate cellular immunity



Deletion mutant

No deletion mutant fish vaccines are commercially available

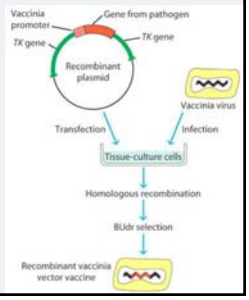
Efficacy: Mimic pathogens and stimulate mucosal immunity and cell-mediated immunity

Safety: Reversion to virulence less likely in genetically engineered vaccines

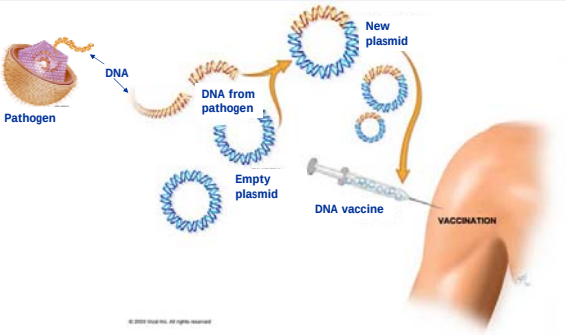
Can be used as marker or DIVA-vaccines (differentiation infected from vaccinated)

Live vectored vaccines

- Efficacy: can be used for viruses with no or poor replication
- Musocal and cellular immunity is stimulated with the right vector
- Safety: Safe, if the vector is non-pathogenic
- Several live vectored vaccines for terrestrial animals, but none for fish



DNA-vaccine - principle



DNA-vaccines

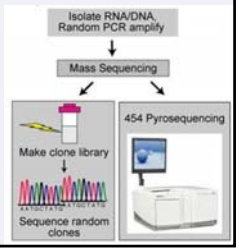
Efficacy: A DNA-vaccine against IHN is licenced in Canada

The vaccine gives acceptable protection

Safety: Fish vaccinated with DNA-vaccines are considered to be gene modified organisms (GMO) in some countries

Pyrosequencing
- tool in vaccinology?

- Heart and skeletal muscle inflammation is a viral disease in salmonid fish
- However, attempts to isolate the virus have so far been unsuccessful
- Sequences of virulence factors can be determined without isolating the microorganism
- The sequence can be expressed in *E. coli* or introduced in a live vector or in a plasmid as a DNA vaccine



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Summary

- Vaccination of aquatic animals is a basis for good health, good economy and sustainability
- Efficaceous vaccines are available for many diseases
- Main challenges for future development are
  - live vaccines
  - oral vaccines
  - vaccines for virus and intracellular bacteria
  - effective adjuvants with minimal side-effects
- Molecular biology is a useful tool for research, production and control of new fish vaccines

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